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Electronic Supporting Information (ESI) for:

Enzymatic synthesis of natural (+)-aristolochene from a non-natural substrate.

Juan A. Faraldos, Daniel J. Grundy, Oscar Cascon, Stefano Leoni, Marc W. van der Kamp, and Rudolf K. Allemann*

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1. Materials. A pre-stained protein size marker (6.5-175) kDa was used to identify proteins by 12% SDS-gel. The Amicon-YM30 membranes were used for protein concentration. For synthetic procedures, all chemicals and solvents were obtained from commercial vendors and used without further purification unless otherwise noted. Anhydrous tetrahydrofuran (THF), diethyl ether, toluene and acetonitrile were obtained from a MBraun SPS800 solvent purification system. Dichloromethane, and triethylamine were distilled from calcium hydride and KOH under nitrogen respectively. EtOH was distilled from calcium oxide.

^1H and ^{13}C NMR spectra were measured on a Bruker Avance 500 NMR spectrometer or a Bruker Fourier300 NMR spectrometer and are reported as chemical shifts in parts per million downfield from tetramethylsilane, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling (to the nearest 0.5 Hz) and assignment, respectively. ^1H , ^{13}C and ^{31}P NMR spectra were measured on a Bruker Avance 500 NMR spectrometer and are reported as chemical shift downfield from tetramethylsilane (^1H and ^{13}C) or 85% H_3PO_4 (^{31}P), coupling constant where appropriate and assignment. Assignments are made to the limitations of COSY, DEPT 90/135, gradient HSQC and gradient HMBC spectra. ^{31}P NMR spectra were recorded on a Jeol Eclipse +300 NMR spectrometer or a Bruker Avance 500 NMR spectrometer.

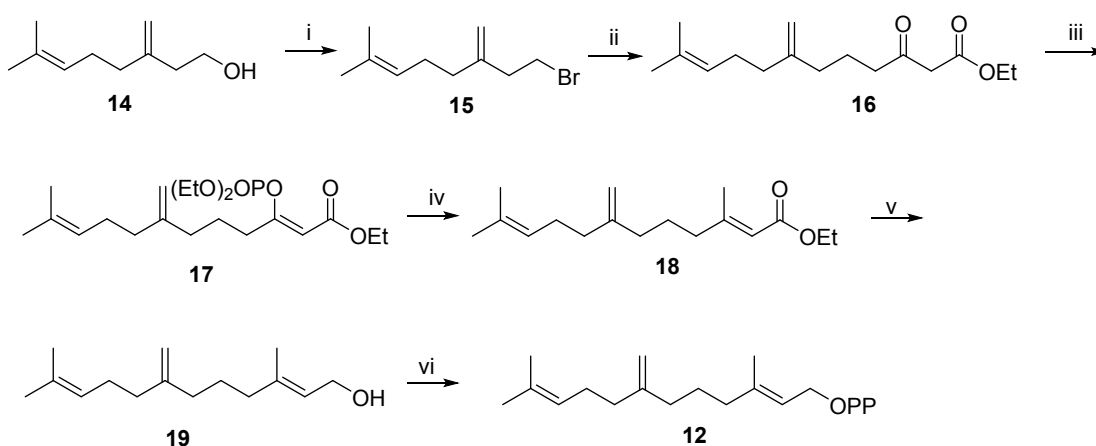
2. General Methods. GC-MS analysis of incubation products was performed on a Hewlett Packard 6890 GC apparatus fitted with a J&W scientific DB-5MS column (30 m x 0.25 mm internal diameter) and a Micromass GCT Premiere detecting in the range m/z 50-800 in the EI^+ mode with scanning once a second with a scan time of 0.9 s. Method 1: The program uses an injection port temperature of 100 °C; split ratio 5:1; initial temperature 50 °C hold 1 min, ramp of 4 °C/min to 150 °C hold 15 min, ramp of 20 °C/min to 250 °C hold 3 min. High-resolution ES^- mass spectra were measured on a Micromass LCT premiere XE spectrometer fitted with a Waters 1525 Micro binary HPLC pump. The purity of purified compounds was judged to be > 95% by TLC and/or GC analyses and NMR spectroscopic analysis. High-resolution ES^- mass spectra were measured on a Micromass LCT premiere XE spectrometer fitted with a Waters 1525 Micro binary HPLC pump.

Thin layer chromatography was performed on pre-coated aluminum plates of silica G/UV₂₅₄. TLC visualizations were performed with 4.2% ammonium molybdate and 0.2% ceric sulfate in 5 % H_2SO_4 , or 0.1 % berberine hydrochloride in EtOH or UV light. Reverse phase HPLC was performed on a system comprising of a Dionex P680 pump and a Dionex UVD170U detector unit.

3. Protein preparation and purification. Recombinant *Penicinium roqueforti* aristolochene synthase¹ (PR-AS), *Solidago canadensis* germacrene A synthase² (GAS) and *Mentha X piperita*

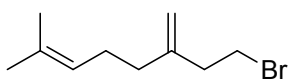
(E)- β -farnesene synthase³ (EBFS) were overproduced in *E. coli* BL21(DE3) and purified as previously described.^{1,2,3} Protein concentrations were determined by the Bradford method using commercial reagents and commercial bovine serum albumin as the calibration standard.^{4,5}

4. Synthesis. (2*E*, 6*E*)-farnesyl diphosphate (**1**) was synthesized from commercial (2*E*, 6*E*)-farnesol using the method described by Poulter.^{6,7} 7-Methylene-farnesyl diphosphate (**12**) was prepared from γ -geraniol (**14**, 7-methyl-3-methyleneoct-6-en-1-ol)⁸ using the established method of chain extension using the acetoacetate dianion.^{9–11} The 7-methylene-farnesol (**19**) was diphosphorylated and purified using the method described by Keller and Thompson,¹² Scheme S1.



Scheme S1. Synthesis of **12**. Reagents and conditions: *i.* Et_3N , MsCl , -45°C then LiBr , RT, 72 %; *ii.* Ethyl acetoacetate, NaH , THF, 0°C , then $n\text{-BuLi}$, followed by **9**, 65%; *iii.* NaH , THF, 0°C , then diethylchlorophosphate; *iv.* Me_2Cu , -78°C , then addition of enolphosphate **17**, -78°C for 2 h, -45°C for 2 h, followed by addition of MeI , -45°C , 0.5 h, 30 % over two steps; *v.* DIBAL-*H*, toluene, -78°C , 85 %; *vi.* MeCN , Cl_3CCN , $(\text{H}_2\text{PO}_4)^{2-}[\text{Et}_3\text{NH}^+]_2$, 37°C , 15 minutes, 45%.

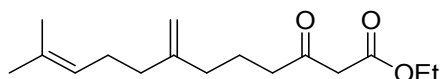
8-Bromo-2-methyl-6-methyleneoct-2-ene **15**



A stirring solution of **14** (600 mg, 3.9 mmol) and Et_3N (2.36 g, 3.25 mL, 23 mmol) in anhydrous THF (50 mL) was cooled to -10°C and methanesulfonyl chloride (893 mg, 603 μL , 7.8 mmol) added dropwise over 5 minutes. The reaction was stirred for a further 30 minutes before lithium bromide (3.4 g, 39 mmol) was added and the reaction allowed to warm to room temperature and stirred for a further 20 h. The reaction was quenched with $\text{d.H}_2\text{O}$ (15 mL) and separated with Et_2O (10 mL). The aqueous layer was further washed with Et_2O (3 x 10 mL) and the combined organic extracts washed with brine, dried (MgSO_4) and the solvent removed under reduced pressure. Purification by column chromatography on silica (5% EtOAc in hexane) yielded the title compound in 72% yield (605 mg, 2.79 mmol).

δ_{H} (300 MHz, CDCl_3) 5.23 – 5.04 (1 H, m, $(\text{CH}_3)_2\text{CCH}$), 4.91 – 4.85 (1 H, m, C=CHH), 4.85 – 4.78 (1 H, m, C=CHH), 3.47 (2 H, t, J 7.5, CH_2Br), 2.58 (2 H, t, J 7.5, $\text{CH}_2\text{CH}_2\text{Br}$), 2.19 – 1.99 (4 H, m, $\text{CCHCH}_2\text{CH}_2$), 1.69 (3 H, d, J 1.0, CH_3), 1.61 (3 H, s, CH_3); δ_{C} (75 MHz, CDCl_3) 146.48 (C=C), 132.18 (C=C), 123.77 ($(\text{CH}_3)_2\text{CCH}$), 111.73 (C=CHH), 39.58, 35.80, 31.17, 26.39 (CH_2), 25.85, 17.89 (CH_3); HRMS (APCI^+ , $[\text{M} + \text{H}]^+$) found 217.0593, $\text{C}_{10}\text{H}_{18}\text{Br}$ requires 217.0593.

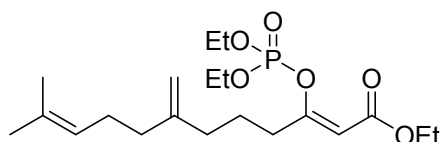
Ethyl 11-methyl-7-methylene-3-oxododec-10-enoate **16**



To an oven-dried RBF charged with a magnetic stirrer bar was added sodium hydride (8.4 mmol, 335 mg, 60% in mineral oil) and washed with anhydrous hexane (3 x 20 mL) by stirring for 5 minutes and removal of the hexane by syringe. The washed sodium hydride was taken up in THF (40 mL) and cooled to 0 °C before the dropwise addition of ethyl acetoacetate (4.2 mmol, 531 μL) over 20 minutes and then stirred at 0 °C for a further 20 minutes. A solution of $n\text{BuLi}$ (4.9 mmol, 1.96 mL, 2.5 M in hexane) was then added dropwise over 20 minutes at 0 °C and the solution stirred for a further 30 minutes. A room temperature solution of **15** (1.4 mmol, 300 mg) in THF (2 mL) was added dropwise over 20 minutes and stirred for 3 h allowing it to slowly warm to 10 °C. The reaction was quenched by dropwise addition of HCl (5 mL, 10% v/v) and stirred for 30 minutes before it was diluted with $\text{d.H}_2\text{O}:\text{EtOAc}$ (1:1, 20 mL) separated and the aqueous layer washed further with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine, dried (MgSO_4) and solvent removed under vacuum. Purification by column chromatography on silica (10% EtOAc in hexane, R_f 0.56 in 20%) gave the title compound in 65% yield (241 mg, 0.9 mmol).

δ_{H} (300 MHz, CDCl_3) 5.15 – 5.05 (1 H, m, $(\text{CH}_3)_2\text{CCH}$), 4.75 (1 H, bs, C=CHH), 4.72 (1 H, bs, C=CHH), 4.19 (2 H, q, J 7.0, $\text{CH}_3\text{CH}_2\text{OC(O)}$), 3.43 (2 H, s, $\text{C(O)CH}_2\text{C(O)OEt}$), 2.54 (2 H, t, J 7.5, $\text{CH}_2\text{C(O)CH}_2\text{C(O)OEt}$), 2.17 – 1.94 (6 H, m, 3 x CH_2), 1.81 – 1.69 (2 H, m, $\text{CH}_2\text{CH}_2\text{C(O)}$), 1.68 (3 H, d, J 1.0, CH_3), 1.60 (3 H, s, CH_3), 1.28 (3 H, t, J 7.0, $\text{CH}_3\text{CH}_2\text{O}$); δ_{C} (75 MHz, CDCl_3) 202.89 (C(O)CH_2), 167.38 (C(O)OEt), 148.67 (C=C), 131.84 (C=C), 124.14 (C=C), 109.84 (C=CHH), 49.55 ($\text{C(O)CH}_2\text{C(O)OEt}$), 35.88, 35.31, 26.48, 25.84, 21.38 (CH_3), 17.86 (CH_3), 14.25 (CH_3); HRMS (APCI^+ , $[\text{M} + \text{Na}]^+$) found 289.1768, $\text{C}_{16}\text{H}_{26}\text{O}_3\text{Na}$ requires 289.1780.

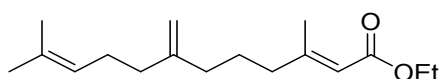
Ethyl (Z)-3-((diethoxyphosphoryl)oxy)-11-methyl-7-methylenedodeca-2,10-dienoate **17**



To an oven dried round-bottom flask charged with a magnetic stirrer bar and flushed with argon was added sodium hydride (256 mg, 6.4 mmol, 60 % dispersion in mineral oil) and washed twice with dry hexane. The washed NaH was taken up in dry Et₂O (30 mL) and cooled to 0 °C while stirring, a room temperature solution of **16** (340 mg, 1.28 mmol) in dry Et₂O (10 mL) was then added dropwise over 15 minutes before being allowed to warm to room temperature and stirred for a further 30 minutes. The reaction was then cooled to 0 °C and neat diethyl chlorophosphate (368 μL, 440 mg, 2.55 mmol) added dropwise over 15 minutes. The reaction was stirred for a further 30 minutes at 0 °C before it was quenched with dropwise addition of sat. NH₄Cl (10 mL) and stirred for 10 minutes (0 °C). The mixture was diluted with H₂O (10 mL), separated, and the aqueous fraction further washed with Et₂O (3 x 10 mL). Combined ethereal extracts were then washed with brine, dried (Na₂SO₄) and solvent removed under reduced pressure, the residue was then stored at -20 °C and used the following day without further purification.

δ_H (300 MHz, CDCl₃) 5.36 (1 H, s, C(O)CHCOP(O)(OEt)₂), 5.16 – 5.00 (1 H, m, (CH₃)₂CCH), 4.76 (1 H, bs, C=CHH), 4.73 (1 H, bs, C=CHH), 4.32 – 4.20 (4 H, m, P(O)(OCH₂CH₃)), 4.15 (2 H, q, *J* 7.0, CH₃CH₂OC(O)), 2.43 (2 H, t, *J* 7.5), 2.16 – 1.96 (6 H, m, 3 x CH₂), 1.78 – 1.69 (2 H, m, CH₂), 1.68 (3 H, d, *J* 1.0, CH₃), 1.60 (3 H, s, CH₃), 1.40 – 1.32 (6 H, m, P(O)(OCH₂CH₃)), 1.27 (7 H, t, *J* 7.0, CH₃CH₂OC(O)); δ_P (122 MHz, CDCl₃) -8.11 (s).

Ethyl (E)-5,13-dimethyl-9-methylene-3-oxotetradeca-4,12-dienoate **18**

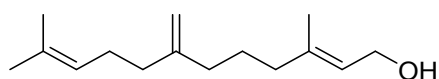


A stirring suspension of copper (I) iodide (495 mg, 2.6 mmol) and Et₂O (30 mL) in an oven-dried round-bottom flask charged with a magnetic stirrer bar and flushed with argon was cooled to 0 °C. Methyl lithium (3.25 mL, 5.2 mmol, 1.6 M in Et₂O) was added dropwise over 10 minutes and stirred at 0 °C for 30 minutes until all the CuI was dissolved to give a clear, almost colourless solution. The solution was then cooled to -78 °C and a solution of **17** (1.3 mmol assumed) in dry Et₂O (7 mL) added dropwise over 30 minutes, a yellow colour developed which then deepened while stirring at -78 °C for a further 2 h. The mixture was then allowed to warm to -45 °C and maintained at this temperature for a further 2 h, over which time the colour darkened and turned a deep purple. Neat methyl iodide (202 μL, 461 mg, 3.25 mmol) was then added and stirred at -45 °C for 20 minutes before the reaction mixture was poured into an ice-cold mixture of sat. NH₄Cl and conc.

NH₄OH (100 mL, 4:1) and stirred for 30 minutes until all the copper salts were dissolved. The mixture was then separated, the aqueous layer further washed with Et₂O (5 x 15 mL) and the combined ethereal extracts washed with NH₄OH (2 x 20 mL, 10 % v/v), H₂O (2 x 20 mL), brine, dried (MgSO₄) and the solvent removed under reduced pressure. Purification by column chromatography on silica (10% EtOAc in hexane, R_f 0.7 in 20%) yielded the title compound in 31% yield over 2 steps (106 mg, 0.40 mmol).

δ_{H} (300 MHz, CDCl₃) 5.67 (1 H, dd, *J* 2.5, 1.0, CCHC(O)OEt), 5.15 – 5.07 (1 H, m, (CH₃)₂CCH), 4.74 (1 H, bs, C=CHH), 4.73 (1 H, bs, C=CHH), 4.14 (2 H, q, *J* 7.0, CH₃CH₂OC(O)), 2.16 (3 H, d, *J* 1.0, CH₃CCHC(O)), 2.18 – 1.97 (8 H, m, 4 x CH₂), 1.69 (3 H, d, *J* 1.0, CH₃), 1.61 (2 H, m, CH₂), 1.61 (3 H, s, CH₃), 1.28 (3 H, t, *J* 7.0, CH₃CH₂OC(O)); δ_{C} (75 MHz, CDCl₃) 149.10 (C(O)OEt), 131.84 (C=C), 124.18 ((CH₃)₂CCH), 115.75 (CCHC(O)), 110.19 (C=CHH), 59.63 (CH₃CH₂OC(O)) 40.64 (CH₂), 35.70 (CH₂), 26.52 (CH₂), 25.85 (CH₂), 25.53 (CH₃), 18.88 (CH₃), 17.86 (CH₃), 14.50 (CH₃CH₂OC(O)). HRMS (EI⁺, [M]⁺) found 264.2090, C₁₇H₂₈O₂ requires 264.2089.

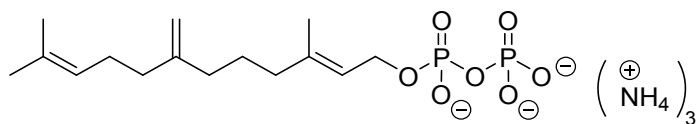
(E)-3,11-Dimethyl-7-methylenedodeca-2,10-dien-1-ol **19**



A stirring solution of **18** (106 mg, 0.40 mmol) in anhydrous toluene (15 mL) was cooled to -78 °C for 15 minutes before the dropwise addition of DIBAL-H (1.2 mL, 1.2 mmol, 1 M in toluene) over 30 minutes. The reaction was stirred at -78 °C for a further 1 h before the dropwise addition of methanol (1 mL) and the mixture allowed to warm to room temperature. A mixture of sat. NH₄Cl and 1 M HCl (1:1, 10 mL) was added and the mixture stirred vigorously for 30 minutes before it was separated and the aqueous layer washed with Et₂O (5 x 5 mL). The combined organic extracts were washed with d.H₂O and brine, dried (MgSO₄) and solvent removed under reduced pressure. Purification by column chromatography on silica (10% EtOAc in hexane, R_f 0.26) yielded the title compound in 85% yield (76 mg, 0.34 mmol).

δ_{H} (300 MHz, CDCl₃) 5.48 – 5.36 (1 H, m, CCHCH₂OH), 5.16 – 5.06 (1 H, m, (CH₃)₂CCH), 4.73 (1 H, d, C=CHH), 4.72 (1 H, d, C=CHH), 4.16 (2 H, d, *J* 7.0, CCHCH₂OH), 2.19 – 1.93 (8 H, m, 4x CH₂), 1.69 (3 H, d, *J* 0.9), 1.67 (3 H, s, CH₃), 1.61 (3 H, s, CH₃), 1.59 – 1.48 (2 H, m, CH₂); δ_{C} (75 MHz, CDCl₃) 149.64, 140.05, 131.75, 124.29 ((CH₃)₂CCH), 123.49 (CCHCH₂OH), 109.07 (CH₂)₂CCH₂(CH₂)₃, 59.56 (CCHCH₂OH), 39.34 (CH₂), 36.16 (CH₂), 35.88 (CH₂), 26.56 (CH₂), 25.90 (CH₃), 25.85, 17.85 (CH₃), 16.34 (CH₃); HRMS (EI⁺, [M – H₂O]⁺) found 204.1879, C₁₅H₂₄ requires 204.1878.

(E)-3,11-Dimethyl-7-methylenedodeca-2,10-dien-1-yl trisammonium diphosphate **12**



Bis-triethylammonium phosphate (TEAP) was prepared immediately prior to use by the dropwise addition of a solution of phosphoric acid in acetonitrile to a stirring solution of triethylamine in acetonitrile. TEAP was then added in three portions (3 x 1 mL) at 5 minute intervals to a solution of **19** (76 mg, 0.34 mmol) in trichloroacetonitrile (1 mL) at 37 °C. The reaction was incubated at 37 °C for a further 5 minutes after the final addition and the entire reaction mixture applied to a silica column and washed onto the column with isopropanol. The mobile phase (isopropanol : conc. NH₄OH : H₂O, 6 : 2.5 : 0.5) was then begun and fractions collected and analysed by TLC (isopropanol : conc. NH₄OH : H₂O, 6 : 3 : 1, visualised with basic KMnO₄), and those containing the diphosphate (*R_f* 0.26) were combined, ammonia and isopropanol removed under vacuum and the resulting aqueous solution diluted to 60 mL with 25 mM NH₄HCO₃ and lyophilised to yield the title compound as a light fluffy powder in 45 % yield (67 mg, 0.15 mmol)

δ_{H} (500 MHz, D₂O) 5.41 (1 H, bs, CCHCH₂OH), 5.15 (1 H, bs, (CH₃)₂CCH), 4.42 (2 H, bs, CCHCH₂O), 2.07 (8 H, bm, 4 x CH₂), 1.66 (3 H, bs, CH₃), 1.64 (3 H, bs, CH₃), 1.60 – 1.48 (2 H, m, CH₂), 1.58 (3 H, bs, CH₃); δ_{P} (202 MHz, D₂O) -6.41 (d, *J* 22.0), -10.21 (d, *J* 22.0).; HRMS (ES⁻, [M – H]⁻) found 381.1215, C₁₅H₂₇O₇P₂ requires 381.1232.

5. Molecular Modeling. To assess similarity in the cyclisation of germacrene A (**3**) and its analogue 7-methylenegemacrene A (**13**), the two molecules were geometry optimized (stationary point search on the potential energy surface) at the DFT/B3LYP level of theory using the standard 6-31G(d) basis set. The calculations were performed using GAUSSIAN09.¹³ The same molecules were optimized by constraining the available volume. To this end, an *ad hoc* van der Waals “corset” was constructed, implemented as a polyhedron of noble gas atoms around the molecules. The encaged molecules were initially geometry relaxed to optimize distances between cage atoms and molecule, followed by a gentle squeeze of the icosahedron to further constrain the space. This step was implemented at finite temperature (Born-Oppenheimer Molecular dynamics with a Nose thermostat set at T= 298 K)) to allow for efficient conformational changes. This was performed using density functional theory as implemented in the SIESTA package.¹⁴ Electronic states were expanded by a double-zeta basis set with polarization functions, constituted of numerical orbitals with a norm-conserving Troullier-Martins pseudopotential description of the core electrons. The Perdew-Burke-Ernzerhof generalized gradient approximation (GGA) to DFT was used. An unconstrained geometry optimization (conjugated gradient search for stationary points) was also performed to confirm agreement of the geometries with the Gaussian results. For both

constrained and unconstrained geometries LUMO molecular orbitals were selected for the sake of illustrating differences in ring closures.

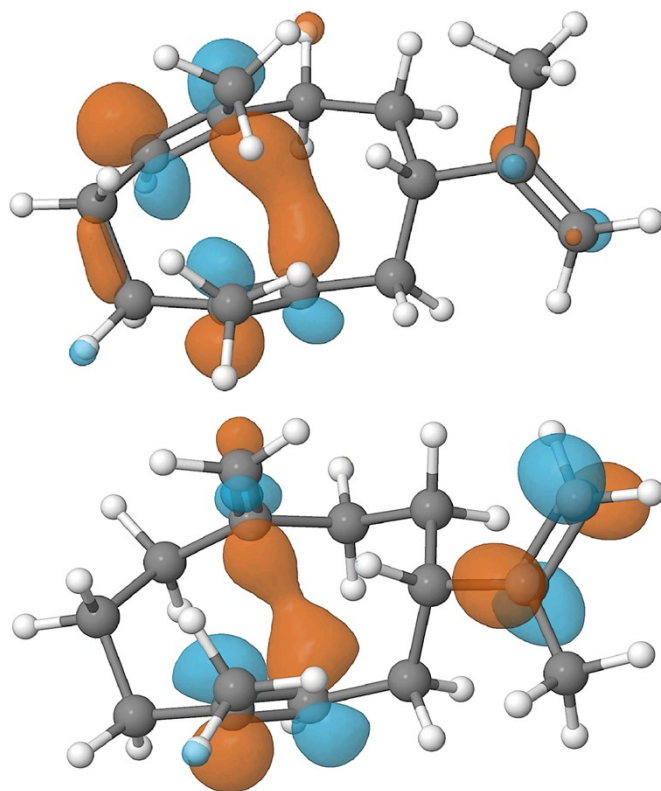


Figure S2. Geometries and LUMOs of **3** (above) and **13** (below) in their reactive UU conformations, after constrained geometry optimization. Under constrained volume conditions **3** and **13** become more similar and the 2,7-contact shorter than the 2,6 contact. Constrained geometries were obtained from DFT/GGA calculations and are very similar to the ones adopted on docking into the enzyme (see also Fig. 1).

To assess the likely positioning in the aristolochene synthase (PR-AS) active site and the influence of the protein environment on the electronic structure, the constrained geometries (see above) were docked into the active site using AutoDock Vina.¹⁵ The protein active site was taken from PDB ID 4KVY chain A,¹⁶ from which the carbocation aza analogue was removed. (This structure is from *A. terreus* AS instead of *P. roqueforti* AS, but these enzymes have 61% sequence identity and comparison of their X-ray structures revealed that they have a very similar overall 3D structure and rely on nearly identical active sites for catalysis.) During docking, the C10-C11 bond was allowed to rotate to adjust to the active site cavity. Top docking poses were selected that had the methyl-groups oriented consistently with the FSPP substrate analogue in PDB ID 4KUX.¹⁶ As docking is performed in the absence of bulk solvent and non-polar hydrogens (and no hydrogen atoms are

present in the crystal structure), a solvent sphere (20 Å around C3 of the docked species) and hydrogens were added using the AmberTools¹⁷ program tleap. (According to PropKa¹⁸, all amino acids were in their standard protonation states. His37 and His132 were singly protonated on Nδ1 and other His singly protonated on Nε2, in line with the hydrogen bonding network.) PP_i was assumed to be singly protonated on the oxygen closest to C12, consistent with its likely role in proton abstraction from the transient germacrene cation. Subsequently, the positions of hydrogen atoms were optimized using energy minimization with the ff14SB¹⁹ protein force-field and TIP3P water model using the AmberTools¹⁷ program sander (25 steps Steepest-Descent and 175 steps Conjugate Gradient).

For constrained and unconstrained geometries and for the constrained geometries in the presence of the protein environment (i.e. in the field of amber ff14SB/TIP3P point charges obtained above), LUMO molecular orbitals were selected for the sake of illustrating differences in ring closures.

6. Analytical incubations. A solution of 1 μM Enzyme and 200 μM isoprenyl diphosphate in incubation buffer (250 μL, 50 mM Tris, 5 mM βME, 5 mM MgCl₂, pH 8.0) was prepared. The aqueous layer was overlaid with HPLC grade pentane (0.5 mL) and the resulting mixture was gently agitated (6 - 18 h) at 25 °C. The incubations were repeated without enzyme as negative controls. The pentane extracts were then analyzed by gas chromatography-mass spectrometry (GC-MS) according to General Methods.

7. GC-MASS SPECTRA

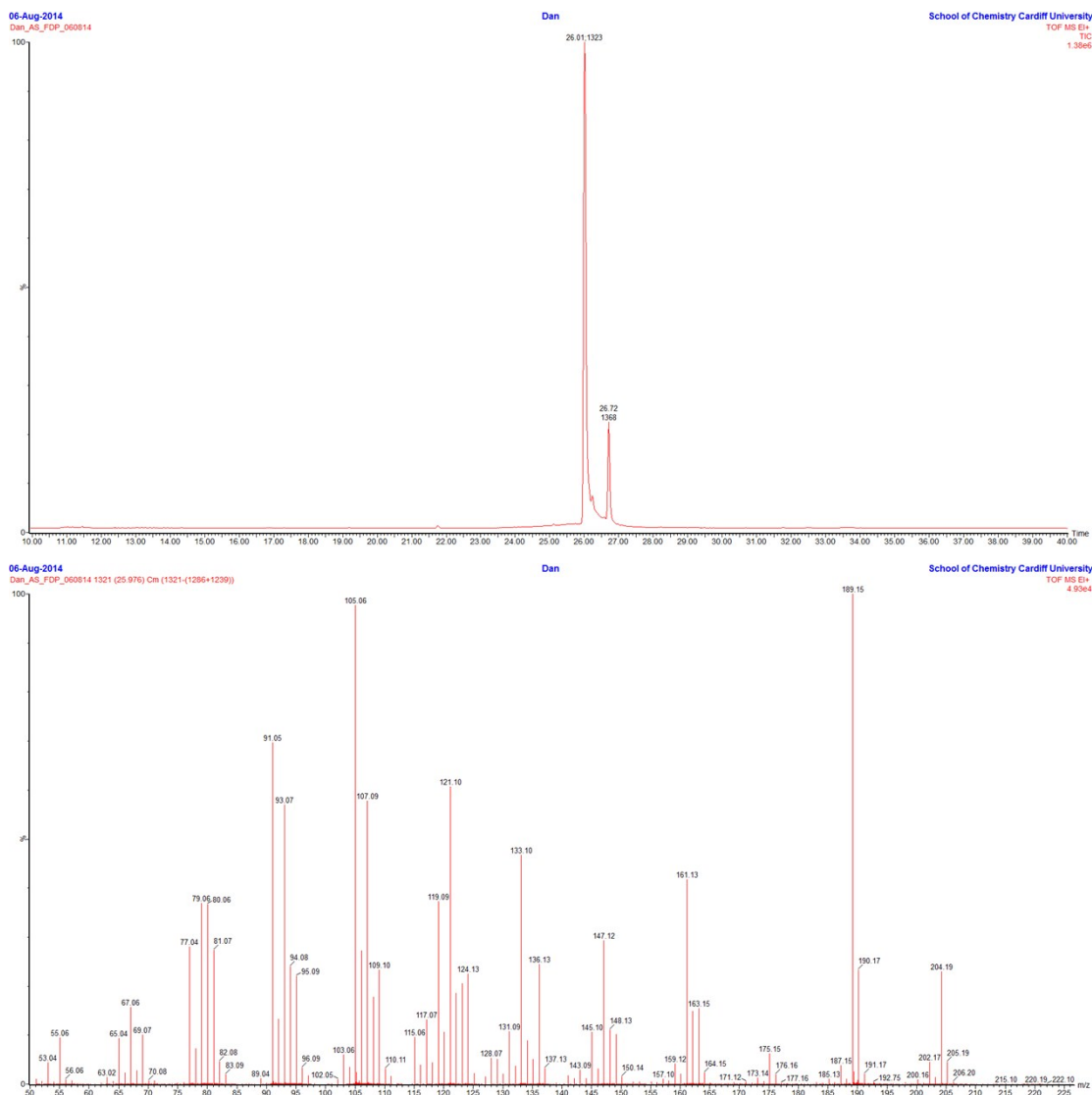


Figure S3. GC-MS analysis (TOF-EI⁺) of the pentane extractable products of an overnight incubation of PR-AS with **1**. Top, Total ion chromatogram and below, mass spectrum (EI⁺) of the compound at 26.01 minutes (Aristolochene).

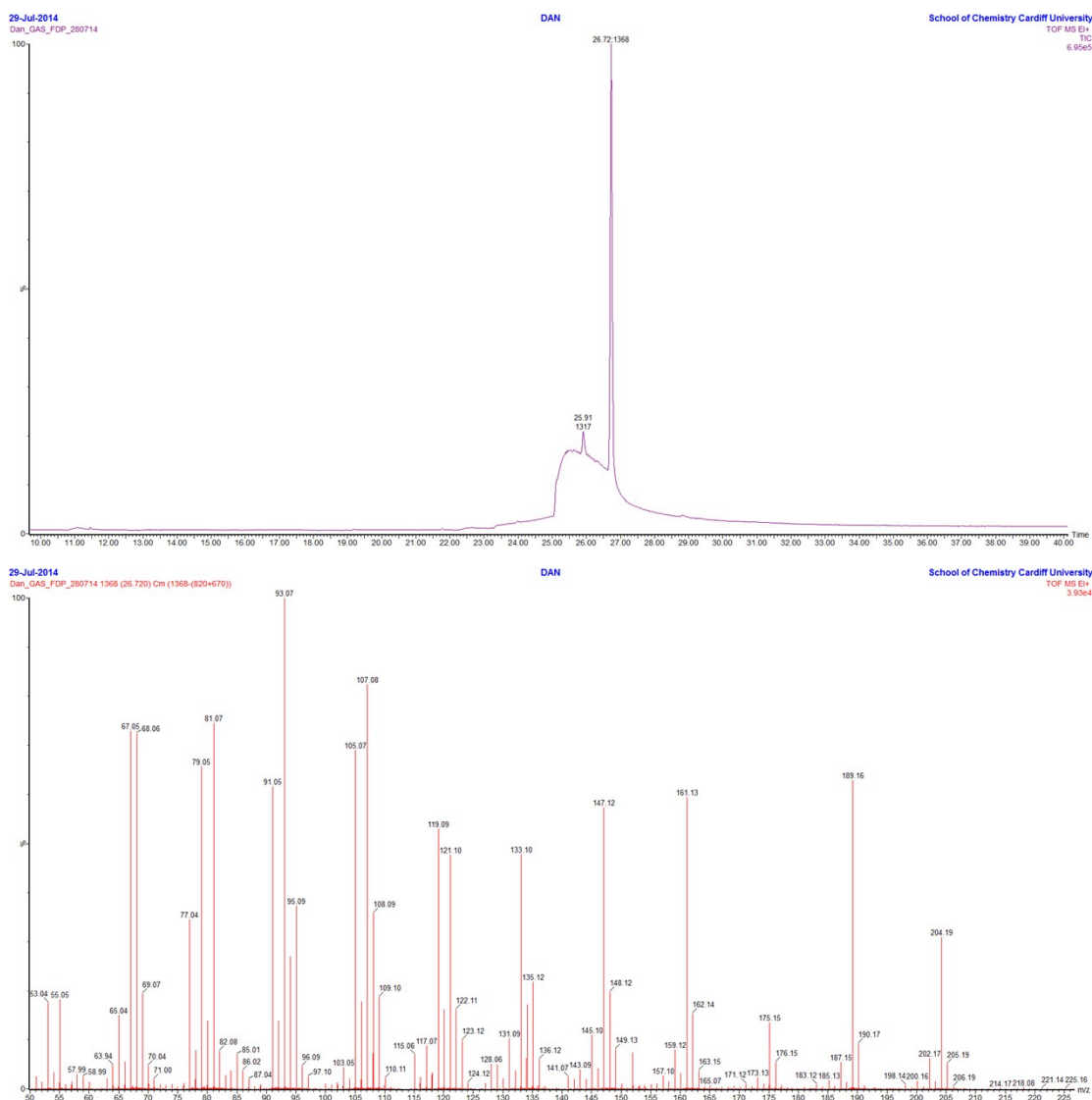


Figure S4. GC-MS analysis (TOF-EI⁺) of the pentane extractable products of an overnight incubation of GAS with **1**. Top, Total ion chromatogram and below, mass spectrum (EI⁺) of the compound at 26.72 minutes (germacrene A, **3**).

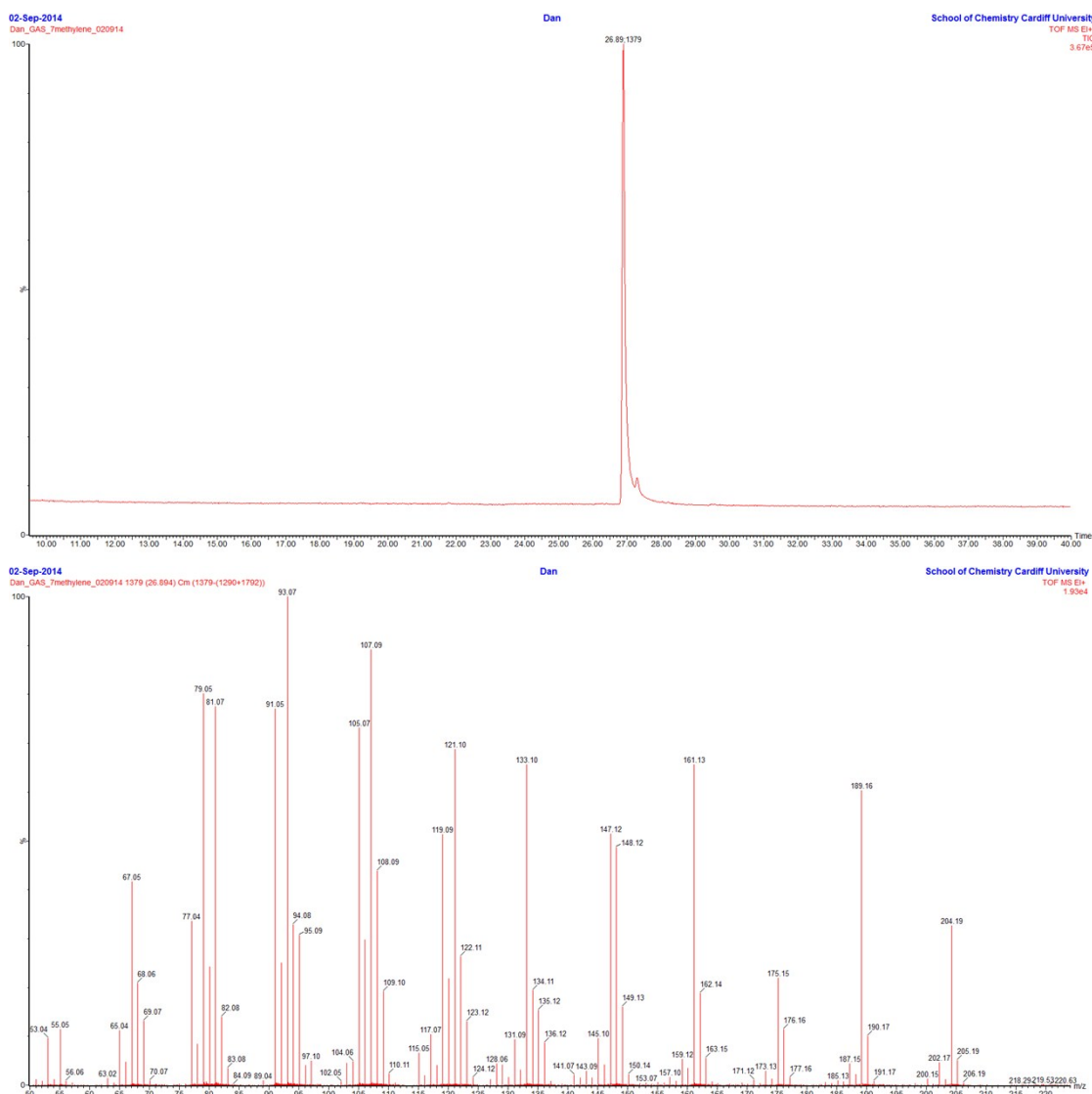
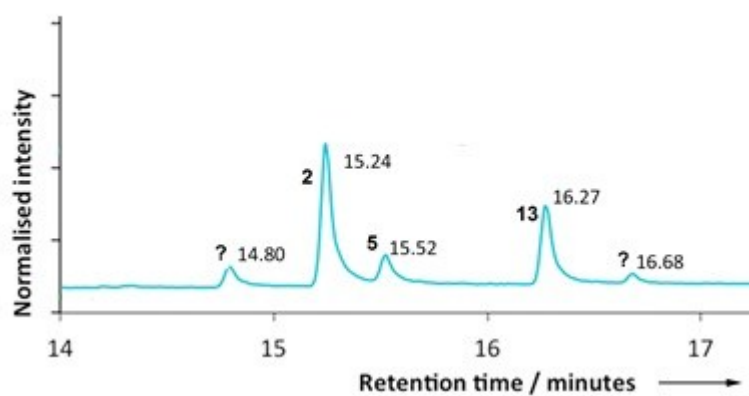
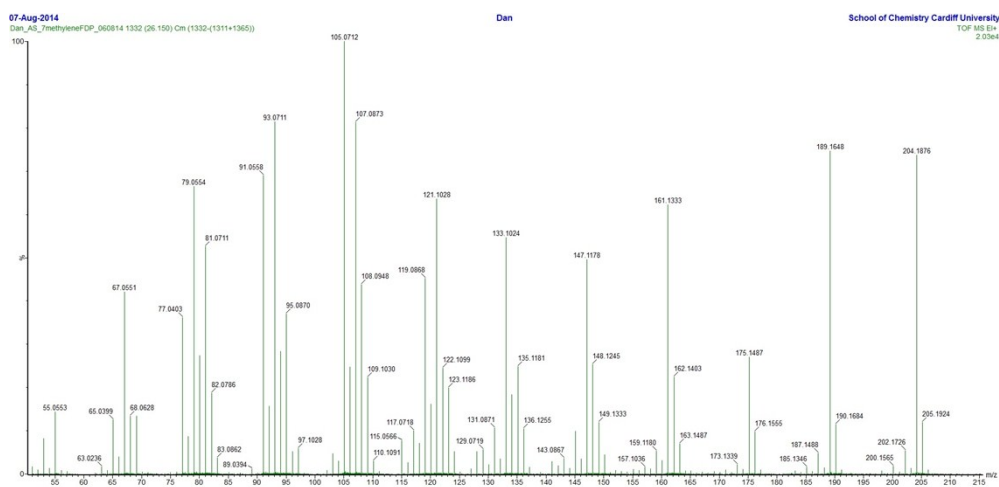
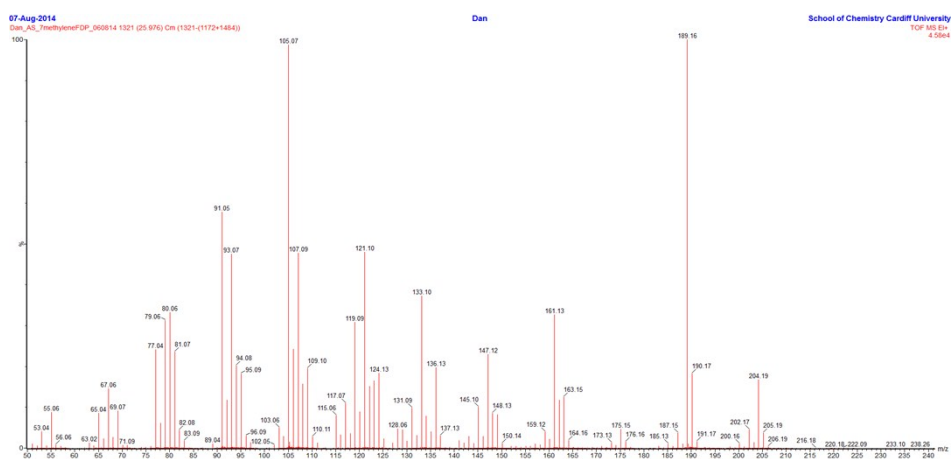
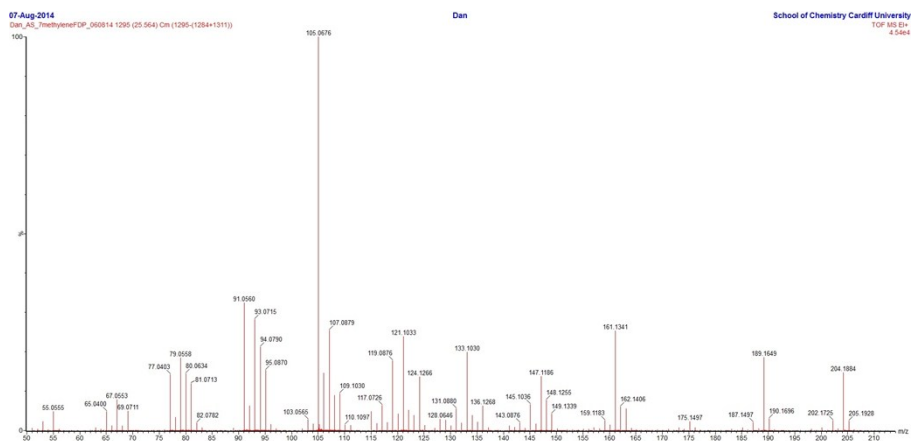


Figure S5. GC-MS analysis (TOF-EI⁺) of the pentane extractable products of an overnight incubation of GAS with **12**. Top, Total ion chromatogram and below, mass spectrum (EI⁺) of the compound at 26.89 minutes (7-methylene-germacrene A, **13**).





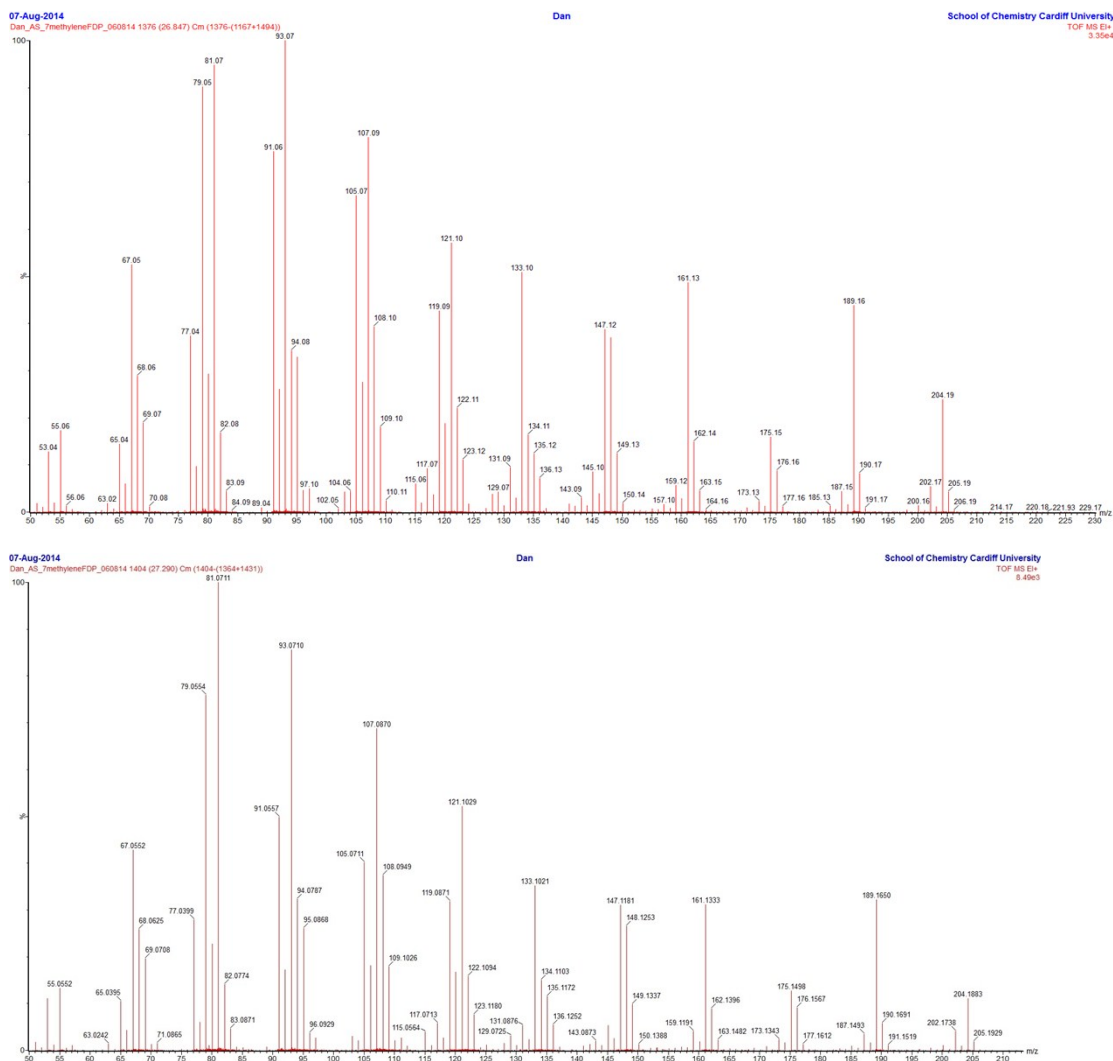


Figure S6. GC-MS analysis (TOF-EI⁺) of the pentane extractable products of an overnight incubation of PR-AS with **12**. Top; total ion chromatogram and below, mass spectrum (EI⁺) of the compounds eluting at 14.80 (unknown), 15.24 (aristolochene, **2**), 15.52 (valencene, **5**), 16.27 (7-methylene-germacrene A, **13**), and 16.68 minutes (unknown).

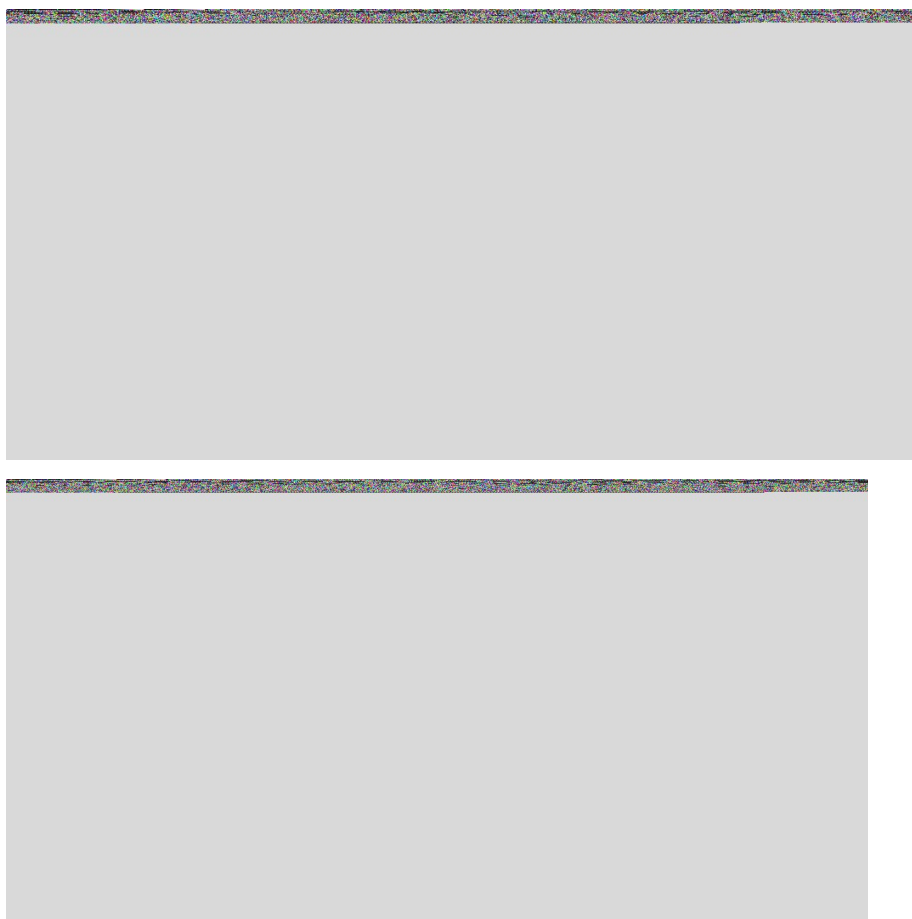
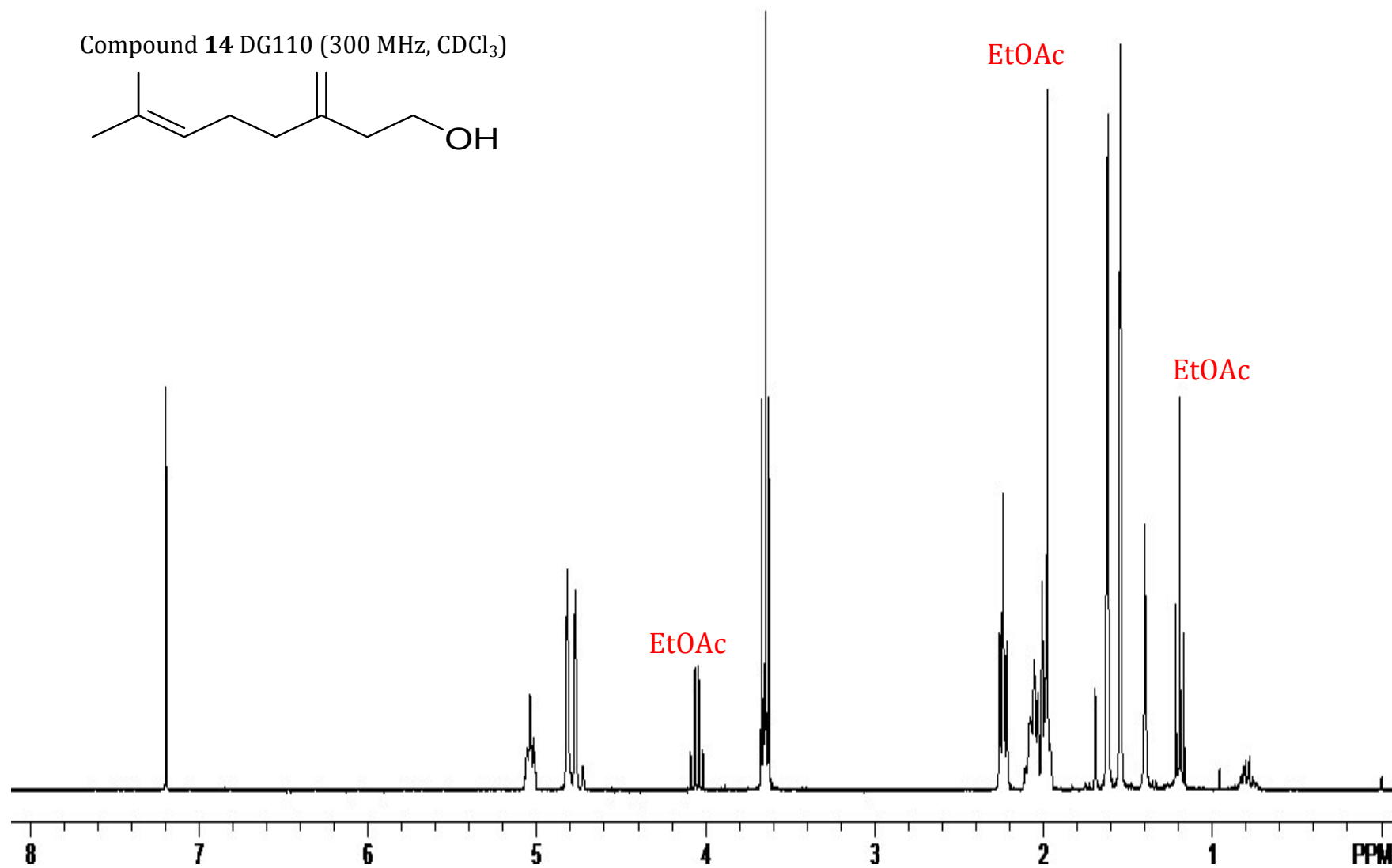
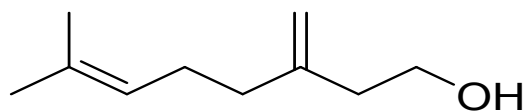


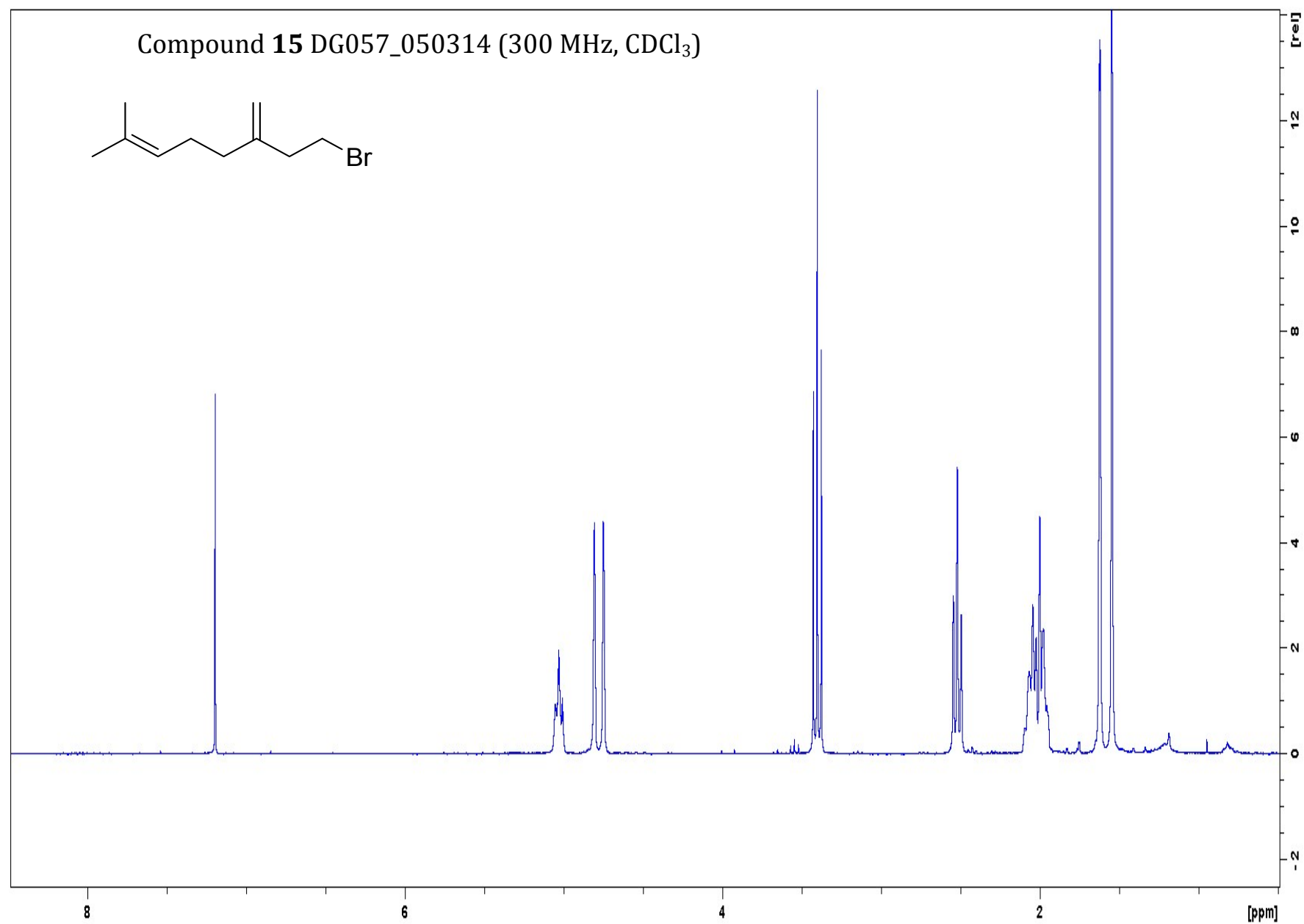
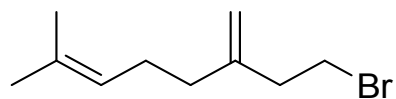
Figure S7. Mass spectra of aristolochene (**2**) obtained from incubations of PR-AS with diphosphates **12** (top) and **1** (bottom)

8. NMR SPECTRA

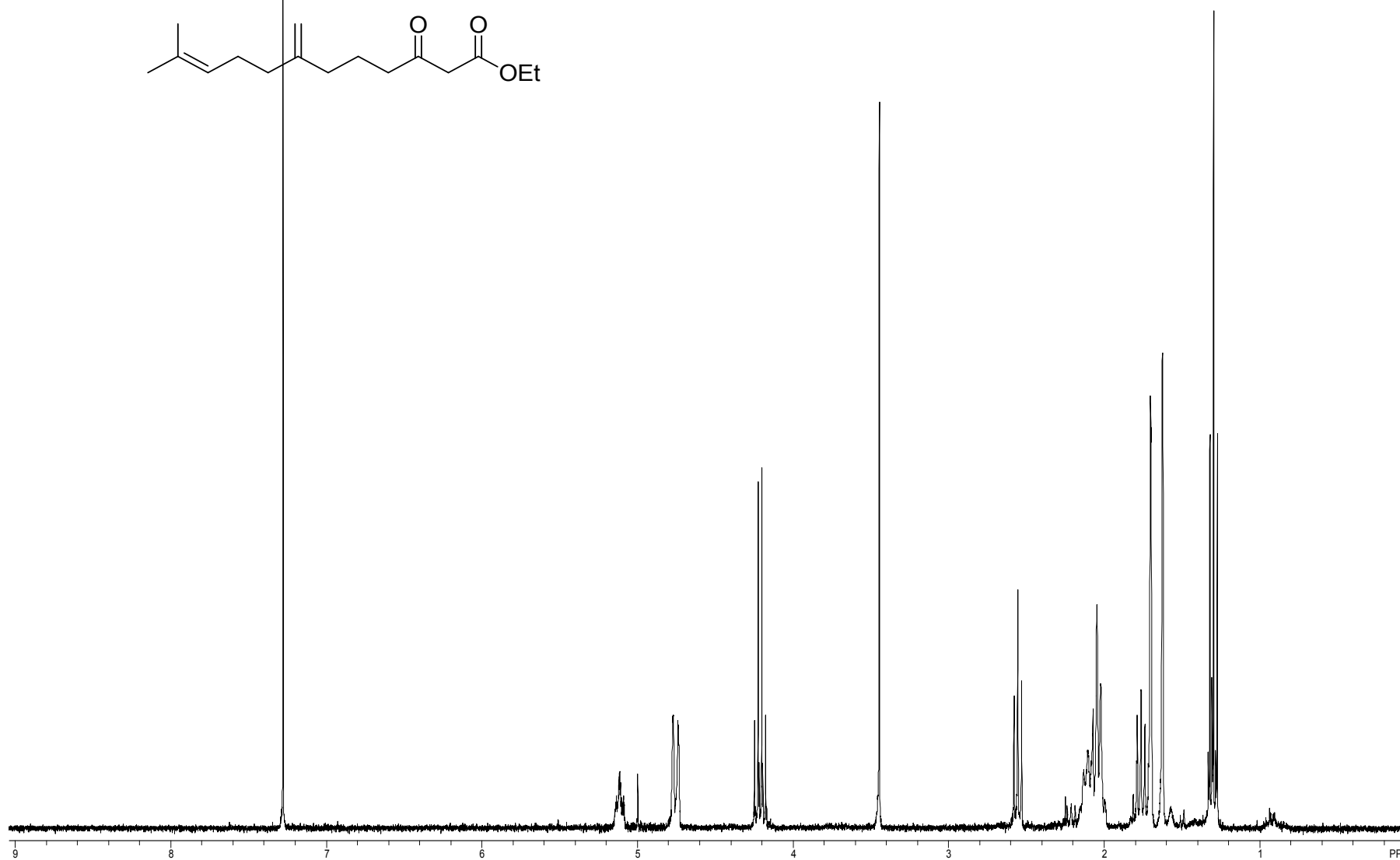
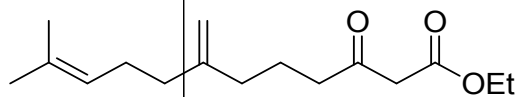
Compound **14** DG110 (300 MHz, CDCl₃)



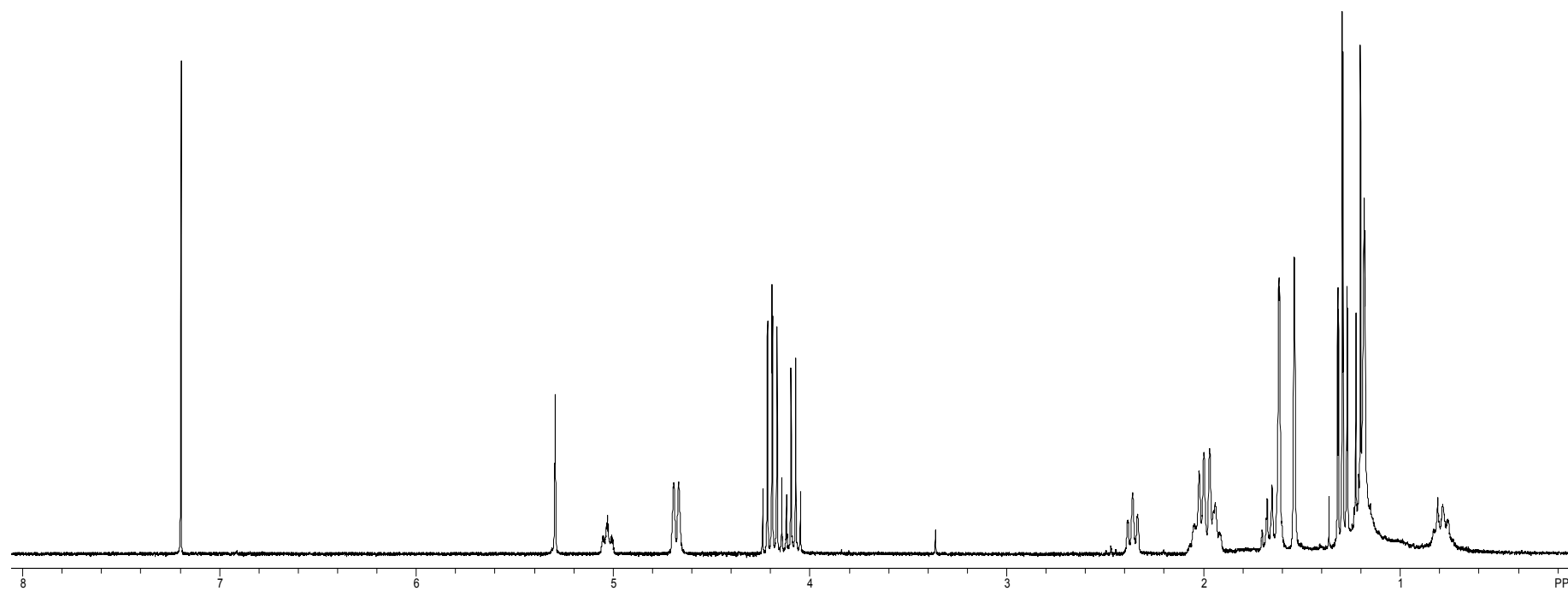
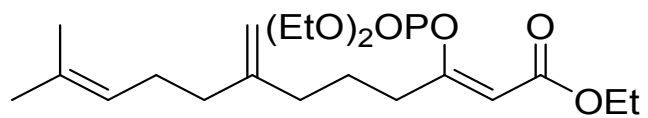
Compound **15** DG057_050314 (300 MHz, CDCl₃)



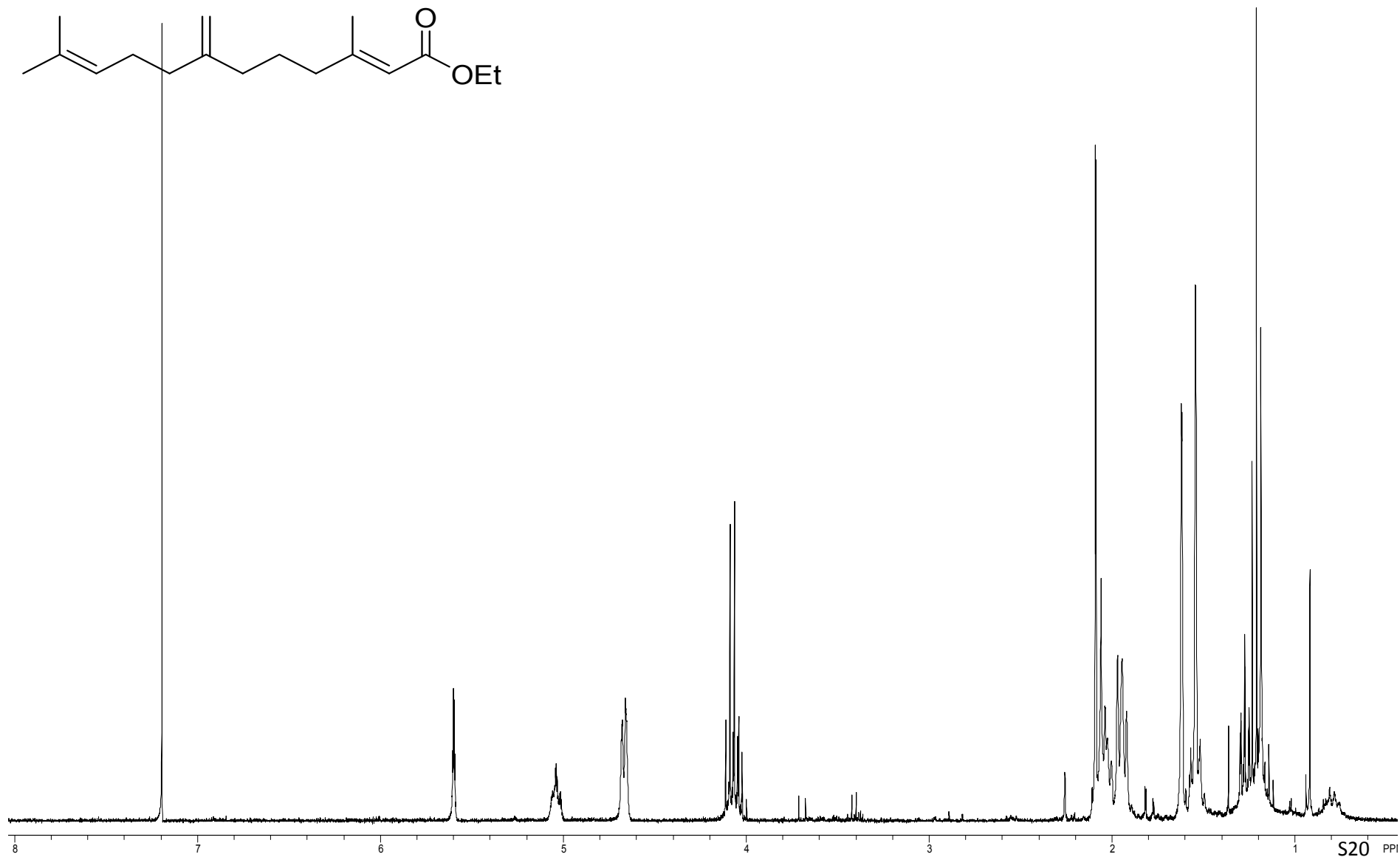
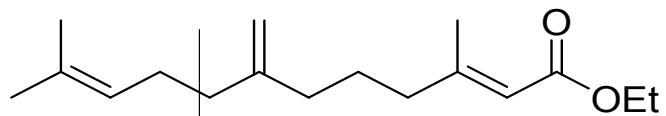
Compound **16** DG119_200514 (300 MHz, CDCl₃)



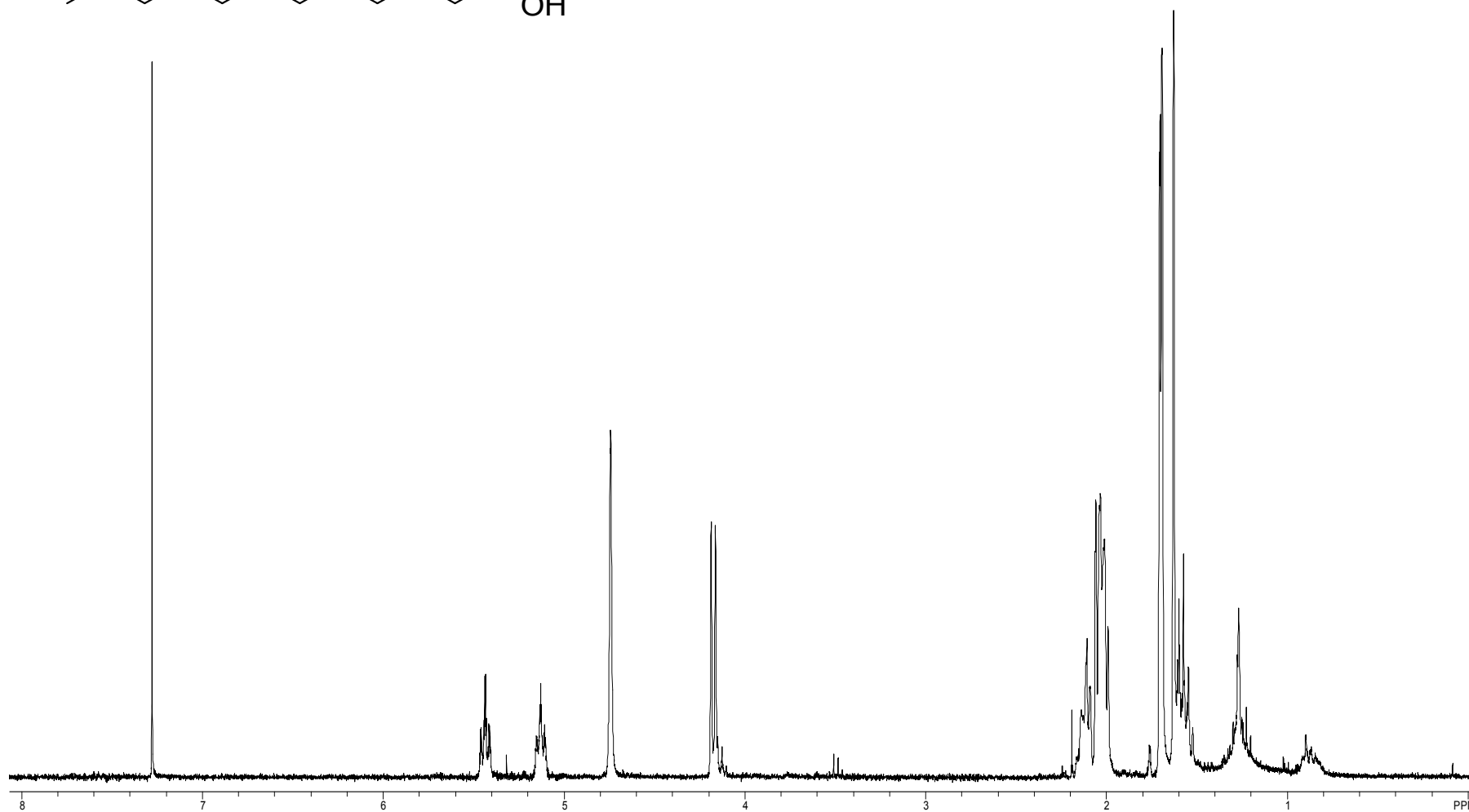
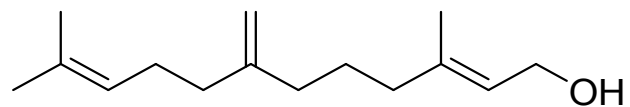
Compound **17** DG150_170914_10 (300 MHz, CDCl₃)



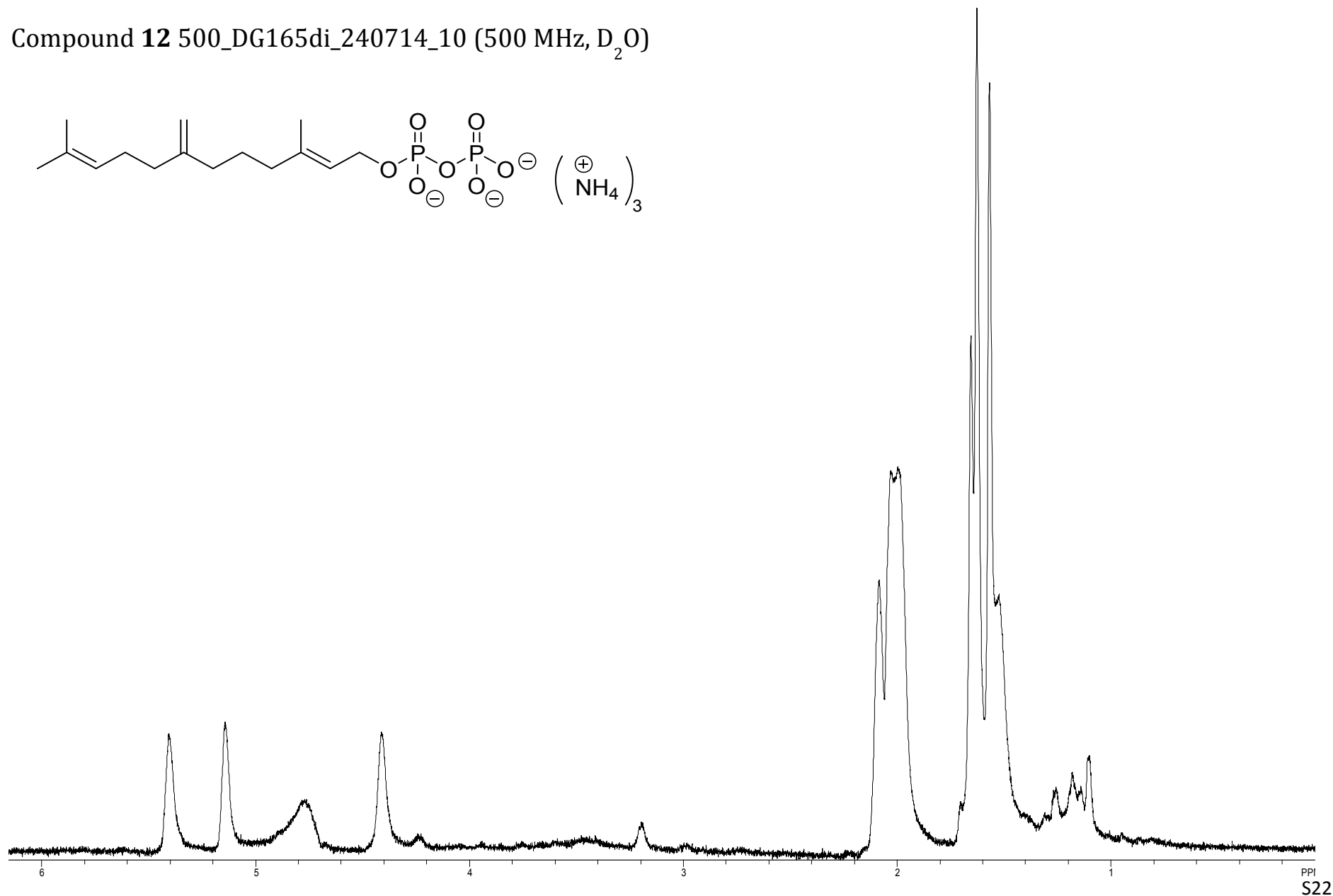
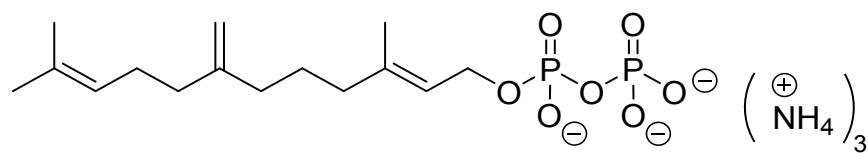
Compound **18** DG150_020714_10 (300 MHz, CDCl₃)



Compound **19** DG161_090914_10 (300 MHz, CDCl₃)



Compound **12** 500_DG165di_240714_10 (500 MHz, D₂O)



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